Comparison of a Chemical and an Electrochemical Enrichment Methods of a Saline Inoculum for Microbial Fuel Cells

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ABSTRACT

In microbial fuel cells (MFCs) efficient extracellular electron transfer microbes, also known as anode-respiring bacteria, play an important role on cell performance. These type of microbes can be developed by application of enrichment procedures. The objective of this study was to compare a chemical (only ***C***, final terminal electron acceptor Fe(III), an electrochemical (only ***E***), and a hybrid method (***E*** followed by ***C***) enrichment methods departing from a saline-sodic soil inoculum. In the electrochemical enrichment procedure in an electrolysis cell, the inoculum was subjected to a continuous electrical stress continually by posing the cell at -150mV/SCE. The only ***C*** enrichment method delivered powers superior to the only ***E*** one (higher values of $P_{An,max}$ = 49 mW/m^2 and $P_{V,max}$ = 558 mW/m^3 of C compared to 33 and 379 of only ***E***). Interestingly, overall resistance as determined by EIS was lower for only E (1240 Ω) than for only ***C*** (1632 Ω). Yet, the hybrid ***E*** method (***E*** followed by ***C*** as given by three serial transfers after the enrichment in the electrolysis cell), showed electrochemical characteristics consistently superior toboth only ***C*** and only ***E*** methods (higher $P_{An,max}$ and $P_{V,max}$, lower internal resistance). Further detailed electrochemical studies of only ***E***-method showed that the anodic resistance decreased with the time of operation of the electrolysis cell that would be consistent with the adaptability/enrichment purpose of the method. Also, Cyclic Voltammetry peaks with values close to those reported for bacterial cytochromes appeared with time of cell operation.

To the best of our knowledge, this is the first time that it is reported that serial transfers with Fe(III) as electron acceptor to an inoculum previously enriched in an electrolysis cell, leads to improved characteristics of microbial fuel cell and increased Fe(III)-reducing capability of the inoculum.

Key words: electrochemical active bacteria, electrolysis, enrichment, exocellular electron transfer, Iron (III), microbial fuel cell

1. Introduction

A microbial fuel cell is an electro-biochemical reactor capable of directly converting organic matter into electricity. In the anodic chamber the microorganisms anoxically oxidize the organic matter and release electrons and protons. Electrons are transported to the anode that acts as an intermediate, external electron acceptor. The electrons flow through an external circuit where there is a resistor or a device to be powered, producing electricity and finally react at the cathode with the protons and oxygen producing water. Microbial fuel cells (MFCs) constitute a promising technology for sustainable production of alternative energy and waste treatment [1].MFCs performance is depends on the internal resistance, cell architecture, type of inocula and some other factors[2,3].

In microbial fuel cells (MFCs) efficient extracellular electron transfer microbes (EETM) also known as anode-respiring bacteria (ARB) can play an important role on cell performance. Selection of the suitable microbes for MFCs by the different redox potentials of electron acceptors, the energetic requirements of a cell vary depending upon the terminal electron acceptor, for example: *(i)* internal electron acceptor (such as fumarate), *(ii)* external electron acceptor such as insoluble Fe (III) and *(iii)* solid electrode with an internal electron acceptor. There is some evidence that the anode potential, rather than the acceptor concentration, regulates the thermodynamic energy available for ARB to grow [4]. It is generally accepted that ARB communities should be capable of switching their respiratory mechanism in order to maximize the energy obtained for ATP production as the anode potential changes. In a mixed ARB community, several respiratory pathways could be available, and the community may be able to maximize energy efficiency by adapting to the anode potential. Torres et al., 2009 used activated sludge as inoculum and found that the two electrodes at the lowest potential showed a faster biofilm growth and produced the highest current densities, reaching a value up to 10.3 A/m^2. Therefore, anodic potential regulation as incisive selective pressure on microbial community can be an important tool for enrichment of efficient ARB community growth.

Cho et al.[5] performed a chemical enrichment by successive cultures of *Shewanella oneidensis* MR-1. Actually the serial cultures implemented with a soluble electron donor (lactate) and acceptors (fumarate) were mainly aimed at preadapting the bacterial metabolism to the anaerobic environment in the MFC. Compared with unadapted bacteria, the anaerobically adapted cells showed improved efficiency in electricity generation of around 30%, even after re-exposure to air. This suggests an adaptation of the microbial population due to metabolic adaptations or genetic mutations [5].

Rabaey et al.[6] have described enrichment by successive transfers of a bacterial consortium from the anodic compartment of an MFC [6]. The biofilm formed on the anode was scratched and used to inoculate a new MFC. During the enrichment period, the power output increased from 0.6 W·m-2 of anode surface to a maximum of 4.31W·m-2. It was concluded that microbial fuel cells enhanced the growth of bacteria that could use the electrode as a final electron acceptor. EETM is likely that combination of multiple enzymes to catalyze the oxidation of carbon

sources such as glucose and transfer electrons to the electrode by: *(i)* direct electron transfer (membrane bound enzymes, bacterial nanowire); *(ii)* indirect electron transfer (self mediator/mediator) [7-10].

It is known that interspecies electron transfer is a key process in methanogenic and sulphate-reducing environments. The objective of this study was to compare three methods of enrichment: chemical (final terminal electron acceptor – Fe(III)), electrochemical (final terminal electron acceptor- solid anode poised with -150 mV vs. Saturated calomel electrode), and a hybrid method (electrochemical followed by chemical) of a saline-sodic soil inoculum from Texcoco lake, Mexico, D.F. The final terminal electron acceptor in the electrochemical method was a solid electrode surface through this more efficient ARB community selected for MFCs applications. An electrochemical tool was used to characterize the ARB community developed at anode surface.

2. Experimental

Sampling for inoculum and enrichment

Samples were collected from the former Texcoco lake in a sterile anaerobic container and preserved aseptically. The sampling site is the dry bottom of a historically desiccated lake in central Mexico; it accumulated the salts in that process and the soils are known to be saline-sodic (Table 1 from [11]) Electrochemically enriched Texcoco soil bacterial community was enriched and preserved in modified Soap Lake basal medium (SLBM) called SL3 medium [12]. This medium was used in enrichment, preservation and electrolysis cell process. The culture was preserved in Refrigerator at -10ºC.

Table 1. Characteristics of Texcoco soil

Site	Salinity	pH	Conductivity of the extract	Texture
Former Texcoco Lake	80-90 g L^{-1}	9.5-11	200 mS cm^{-1}	Sandy clay loam

Electrochemical set-up and electrode preparation

The working (geometrical area- 14.05 cm^2) and counter electrodes (geometrical area- 20.5 cm^2) were Graphite rods. A saturated calomel electrode was used as a reference electrode. The modified SL3 medium with 15mM of sodium acetate was used as carbon source. Potentials were applied with a 273A Potentiostat/Galvanostat from EG&G Princeton Applied Research. Temperature was set at 30ºC. Graphite rods were submerged in 0.5 M KCl solution for 3 h, after that the graphite rods were polished with 1500b sand paper and rinsed with deionized water before use. The graphite rods were submerged in 0.5 M KCl solution overnight in order to activate them. In order to selectively grow the electro active biofilm of ARB, a potential step of -150 mV was applied over 150 day. During this period, the current was monitored. Later on the biofilm was sup cultured using SL3 medium.

Chemical (*C*) and electrochemical (*E*) method of enrichment

Initial inoculum of Texcoco soil was incubated in 1g of soil for Chemically activated Halo alkaliphilic bacteria: 1g of

biofilm scarped from the bioelectrolysis cell for electrochemically active halo alkaliphilic bacteria into 99 ml modified SL3 medium for both chemically and electrochemically activated Halo alkaliphilic bacteria with 15mM of sodium acetate used as carbon source and 20 mM iron (III) citrate as terminal electron acceptor, After the incubation period of 12 days taken the 1 ml of above culture solution was transferred into fresh 99 ml modified SL3 medium (serial transfer I) . Afterwards, similar cultures every 12 days time intervals up to serial transfer III (hybrid ***E***) (Fig 1.). Iron reduction and SCMFC characteristics were evaluated using inoculum of each serial transfer.

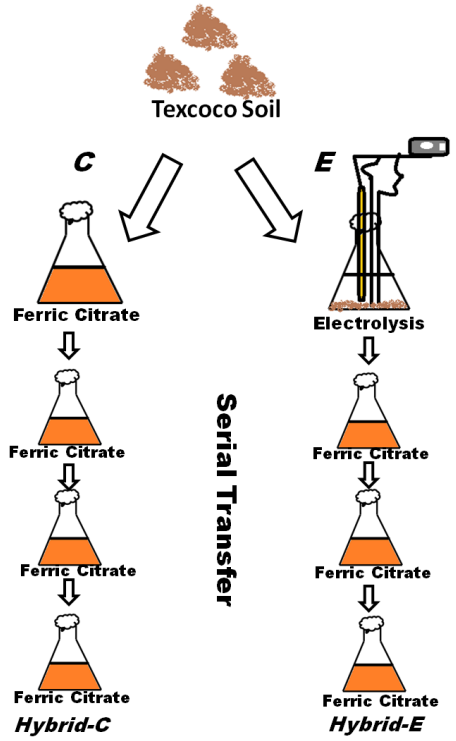

Figure 1. Schematic representation of the ***C*** and ***E*** enrichment serial transfer

Vertical single-chamber microbial fuel cell (SCMFC)

Construction of single chamber microbial fuel cell (SCMFC) details can be found elsewhere [13]

Electrochemical impedance spectroscopy and linear sweep voltammetry studies

Impedance spectra of biofilm were obtained at the open circuit potential (Eocp). The amplitude of the signal perturbation was 10 mV, the frequency range scanned was from 100 kHz to 1 mHz. Impedance experiments were performed in the potentiostat/galvanostat Volta lab model PGZ402. Data fitting was accomplished by appropriate software, such as Z-view. Liner sweep voltammetry (LSV), was run at the recommended scan rate of 1mV s-1 starting from the measured open circuit potential [14] using a 273A Potentiostat/Galvanostat from EG&G Princeton Applied Research.

Cyclic voltammetry

Cyclic voltammetry (CV) studies were performed on the SCMFC with a PARSTAT 2273 Potentiostat/Galvanostat from Princeton Applied Research. The working electrode was the anode. The reference electrode was a standard calomel electrode (SCE) that was in contact with the cell liquor through a saline bridge known as Luggin capillary tube placed closely to the working electrode. The counter electrode was the cathode. The scan rate was 15mV/s.

Most probable number of iron reducing bacteria

Initial inoculum of Texcoco soil was incubated in 1g of soil for Chemically activated Halo alkaliphilic bacteria: 1g of biofilm for electrochemically active halo alkaliphilic bacteria into 99 ml modified SL3 medium for both chemically and electrochemically activated Halo alkaliphilic bacteria with 15mM of sodium acetate used as carbon source and 20 mM iron (III) citrate as terminal electron acceptor, set to be 10^{-1} dilution and 10 ml of above inoculum transferred into 90 ml of SL3 medium set to be 10^{-2} dilution, from that 10 ml of inoculum transferred into 90 ml of SL3 medium as 10^{-3} dilution. Similarly continue the dilution up to 10^{-5} dilution. After the incubation period (~12 days) it was performed the iron reduction test in a plastic well using ferrozine technique [15].

HPLC and UV-Vis spectrophotometer analysis

Secondary metabolism of E-HAB in SCMFC was analysed through HPLC. UV-Vis Spectrophotometery was carried in the wavelength of 200-800nm.

Protein estimation and Sodium Dodecylsulfate Poly(acrylamide) Gel Electrophoresis (SDS-PAGE) Experiments

Protein was precipitated by the trichloroacetic acid (TCA) method [16] from the supernatant solution of the liquor in the SCMFC. Bradford method was used to estimate the protein [17]. SDS-PAGE experiments were run with electrophoresis unit of Bio-Rad [16].

3. Results and discussion

Enrichment of electrochemical active halo alkalophilic bacteria (E-HAB) was performed by variation of current intensity during biofilm formation at -150mV vs SCE as shown in Fig.2a. During the first 5 days, the recorded current was very small. After 5 days, the current started to increase and attained the maximum value of 1.8 mA in the 28th day. Subsequent to 30 days the current decreased down to 0.4mA. Addition of carbon source at 75th day lead to an increase in the current up to 0.8mA in at 135th day (Fig2a). Variation of current intensity during biofilm formation at -150mV vs SCE resembled the bacterial sigmoidal growth curve; the latter is in agreement with previous reported

works [18]. During the first 5 days, the growth of microbial colonies was observed on the graphite rod (anode) in the log phase related to low current. Following 5 days, the biofilm ARB grew and attained the maximum current in the 28th day, probably due to attaining the stationary phase. The depletion of substrate probably lead to the microbial colonies to decline phase of growth, that started in the 30th day. By adding the carbon source at the 75th day the bacterial community started to grow again; this was reflected by the current increment and attained 0.8mA in the stationary phase.

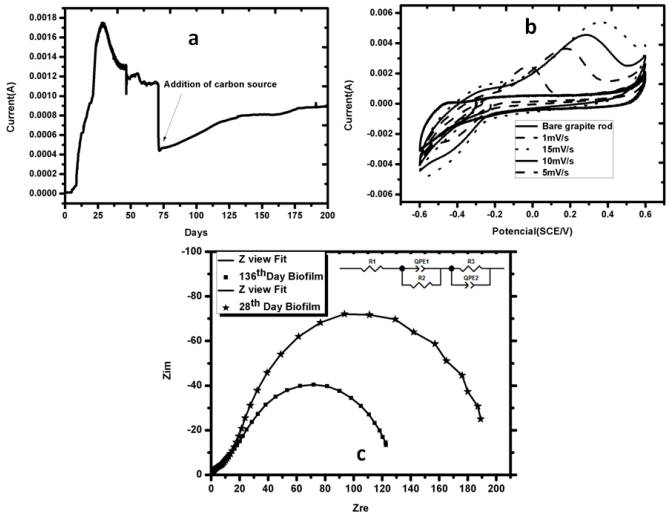

Fig. 2 (a). Variation of Current (A) during biofilm formation at -150mV vs saturated calomel electrode. (b). Cyclic voltammetry of initial GR at 15mV/s. the cyclic voltammetry of biofilm in bicarbonate buffer (0.1M) with 15mM of sodium acetate as carbon source were measured at different scan rates. (c). Electrochemical impedance spectroscopy of biofilm electrode.

Cyclic voltammetry (CV) experiments were performed for further investigate the electrochemical character of the biofilm. In the initial 4 days, the cyclic voltammograms showed no peaks, presumably due to the fact that the biofilm has not been completely formed (results not shown). CV was performed at the 28th day at a different scan rate upto 15 mV/s. Midpoint potential deduced from the cyclic voltammetry, was +108mV vs standard hydrogen electrode (SHE). This value is close to the alkaliphilic cytochromes potential range [19]. Concomitantly, it could be related to the soluble/membrane-bound cytochromes from alkaliphilic bacteria [19]. By increasing the scan rate the anodic peak shifted to more positive values (Fig. 2b). Even though there was an anodic peak, the corresponding cathodic peak did not provide the same area under the anodic curve (anodic charge). This suggests that an irreversible process is occurring. A similar pattern occurred at the 136th day (data not shown). One of the possible reasons for the irreversible process would be fouling of the electrode surface by strong irreversible adsorption of chemicals [20], or to an increased thickness of the biofilm. Membrane bound enzymes (cytochrome) of biofilm is required to be independent of the electroactive ARB`s metabolism [21].

Electrochemical impedance spectroscopy (EIS) at 28th day and 136th day revealed two semicircles (Fig. 2c). The impedance spectra at 28th day and 136th day were fitted to an appropriate equivalent circuit (EC). A simple EC used

for describing the electrochemical properties of the ARB biofilm is shown in Fig. 2(c). The obtained values of the resistance of the film (R2) were 11.1Ω for the 28th day and 5.5 Ω for the 136th day. From the EIS, the high frequency semicircle could be associated to ARB biofilm electrical properties. The low frequency semicircle was probably linked to the processes occurring at the biofilm/solution interface. The tendency of biofilm resistance was decreased with time of operation. This may be due to the adaptability / enrichment of electroactive bacteria on the surface of the graphite rod which may reduce the internal resistance.

Comparison of of the enrichment methods is shown in Tables 2 and 3. When comparing the ***only C*** (results of 3rd transfer in Table 2) and ***only E*** methods (results of 0 transfer in Table 1), ***only C*** was superior with higher values of $P_{An,max}$ = 48.5 and $P_{V,max}$ = 558 of ***C*** compared to 33 and 379 of ***E***. Yet, the overall resistance as determined by EIS was also lower for ***only E*** (1240 Ω) than for ***only C*** (1632 Ω) (Table 3). When considering the ***hybrid E*** method (***E*** followed by ***C*** as given by the three serial transfers), the electrochemical characteristics ot the ***hybrid E*** were consistently superior to ***only C*** (higher $P_{An,max}$ and $P_{V,max}$, lower internal resistance).

Table 2. Serial transfer studies for enrichments of alkaliphilic inocula for a single chamber microbial fuel cell.

Serial transfer	Enrich-ment metod	Concentration of Fe(II) (mM)		Most Probable Number	$P_{An,max}$ (mW/m^2) $P_{V,max}$ (mW/m^3)
		Initial	Final		
0	***E***	6.2±0.1	31.3±0.6	6148±2	33.0±0.6 378.9±1.1
	C	4.7±0.02	22.7±0.3	8500±15	16.0±0.3 183.7±0.7
I-Serial Transfer	***E***	8.5±0.3	46.8±0.7	5144±5	42.5±1.0 488.9±4.7
	C	6.2±0.5	36.9±0.8	5144±5	31.9±0.6 367.3±0.8
II-Serial Transfer	***E***	13.0±0.5	78.2±1.1	3450±1	52.5±1.2 603.5±0.6
	C	10.3±0.5	69.6±0.3	6148±1	37.4±0.4 430.0±1.5
III-Serial Transfer	***E***	16.0±0.8	79.8±0.7	3399±1	81.2±0.6 934.2±1.8
	C	14.3±0.2	70.1±1.0	4012±2	48.5±1.0 557.6±1.1

Iron reducing activity of both ***E*** and ***C***-enriched inocula increased with the transfer number (Table 2). In general, serial transfer studies of iron reduction activity showed improved characteristics of microbial fuel cell, in agreement with findings of Cho and Ellington [5] and Rabaey et al. [6]. Unexpectedly, MPN of Fe(III)-reducing bacteria was reduced by 40 to 50% of the initial amount for the last transfer in both enrichment methods (Table 2).

Table 2. Electrode and membrane/electrolyte resistances of the MFC by EIS with enriched inocula

Serial Transfer	Enrich-ment metod	R_a	R_c	R_m	$R_{int}=R_a+R_c+R_m$
0	*E*	1098±2	534±1	0.86±0.01	1632±2
	C	1292±2	580±1	0.83±0.03	1872±2
I	*E*	981±1	152±1	0.69±0.02	1132±2
	C	1152±3	468±2	0.86±0.05	1620±1
II	*E*	874±2	209±1	0.41±0.04	1083±2
	C	1081±4	471±1	0.79±0.02	1552±4
III	*E*	620±1	227±2	0.53±0.05	837±1
	C	977±2	263±1	0.65±0.03	1240±1

The supernatant solution collected from the SCMFC was qualitatively analyzed through HPLC technique. It revealed the presence of acetic acid, butyric acid, ethanol, and propionic acid. This is may be associated to mixed microbial flora respiration end products. Afterwards, fresh SL3 medium was added to the SCMFC which was already seeded with the biofilm of the sodic-saline inocula. Then, cyclic voltammetry was performed. Fig.3. shows the voltammogram of the bare carbon cloth and biofilm formed on carbon cloth seeded with E-HAB in SCMFC.

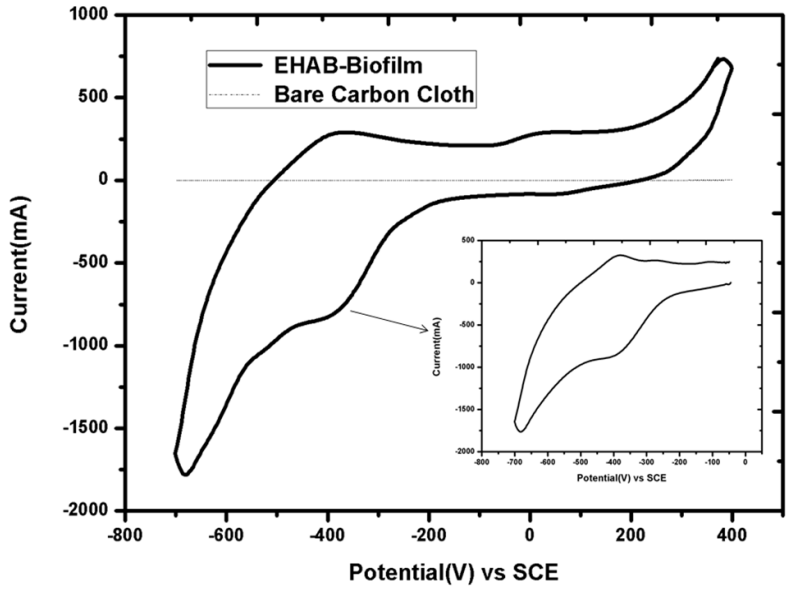

Figure 3.Cyclic voltammetry of the bare and biofilm formed carbon cloth at the scan rate of 15mV/s

The bare carbon cloth did not show any electrochemical response from which we conclude that there is no current generation in the absence of microflora in the SCMFC. Biofilm formed on carbon cloth exhibited two redox

reactions on the surface with midpoint potentials of -385mV and 18mV vs SCE, respectively. The midpoint potential of -385mV might be associated with membrane bound enzymes of the sodic-saline micro-flora, whereas the potential 18mV/SCE could be associated to the soluble cytochrome activity in agreement with results previously reported [22].

The protein content of the liquor in the SCMFC was 363µg/mL. The supernatant solution from E-HAB in the SCMFC contained a protein with a molecular weight ~45kDa as shown by SDS-PAGE (Fig. 4). It may resemble the soluble excreted cytochrome responsible for the midpoint potential of 18mV. The direct electron transfer could be achieved through the cytochrome (membrane bound/soluble excreted), fostering the communication/charge transfer between the electrode and microbes.

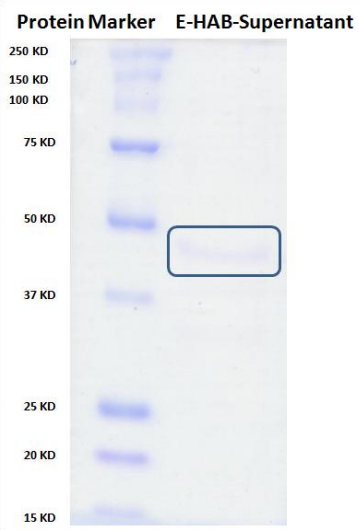

Figure 4. SDS-PAGE analysis of EHAB supernatant solution from SCMFC

Supernatant solution from E-HAB in the SCMFC exhibited a small absorption shoulder at 410 nm (Fig. 5). This was in accordance with the oxidized state of the cytochrome reported by Tomlinson and Ferguson [23].

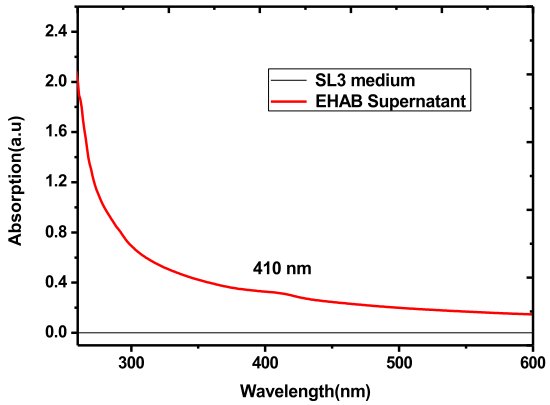

Figure 5. UV-Vis Spectrophotogram of the pure SL3 medium and supernatant solution from SCMFC

4. Conclusion

The ***only C*** enrichment method was superior to the ***only E*** one (higher values of $P_{An,max}$ = 49 and $P_{V,max}$ = 558 of ***C*** compared to 33 and 379 of ***only E***) whereas overall resistance as determined by EIS was lower for ***only E*** (1240 Ω) than for ***only C*** (1632 Ω).

Yet, if we consider the ***hybrid E*** method (***E*** followed by ***C*** as given by the three serial transfers), the electrochemical characteristics of the ***hybrid E*** were consistently superior to ***only C*** (higher $P_{An,max}$ and $P_{V,max}$, lower internal resistance).

Detailed electrochemical studies of ***only E***-method showed that the anodic resistance decreased with the time of operation of the electrolysis cell, that would be consistent with the adaptability/enrichment purpose of the method. Also, CV peaks with values close to those reported for bacterial cytochromes appeared with time of cell operation.

In general, serial transfer studies with Fe(III) as electron acceptor showed improved characteristics of microbial fuel cell and increased Fe(III)-reducing capability of inocula.

Analysis from the Cyclic voltammetry, SDS-PAGE, and UV-Vis Spectrophotometry revealed that the cytochrome (membrane bound/ soluble excreted) might play a vital role in the direct electron transfer in the SCMFC seeded with E-HAB inocula.

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Notation

ARB	anode respiring bacteria
C	chemical enrichment method
CV	cyclic voltammetry
E	electrochemical enrichment method
EIS	electrochemical impedance spectroscopy
HAB	halo alkalophilic bacteria
Hybrid-***E***	serial transfer III of *E*
SCMFC	single chamber microbial fuel cells
SDS-PAGE	sodium dodecylsulfate poly(acrylamide) electrophoresis